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Schizophrenia-associated Chromosome 11q21 Translocation: Identification of Flanking Markers and Development of Chromosome 11q Fragment Hybrids as Cloning and Mapping Resources

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Summary

Genetic linkage, molecular analysis, and in situ hybridization have identified TYR and D11S388 as markers flanking the chromosome 11 breakpoint in a large pedigree where a balanced translocation, $t(1;11)(q43;q21)$, segregates with schizophrenia and related affective disorders. Somatic cell hybrids, separating the two translocation chromosomes from each other and from the normal homologues, have been produced with the aid of immunomagnetic sorting for chromosome 1- and chromosome 11-encoded cell-surface antigens. The genes for two of these antigens map on either side of the 11q breakpoint. Immunomagnetic bead sorting was also used to isolate two stable X-irradiation hybrids for each cell-surface antigen. Each hybrid carries only chromosome 11 fragments. Translocation and X-irradiation hybrids were analyzed, mainly by PCR, for the presence of 19 chromosome 11 and 4 chromosome 1 markers. Ten newly designed primers are reported. The X-irradiation hybrids were also studied cytogenetically, for human DNA content, by in situ Cot1 DNA hybridization and by painting the Alu-PCR products from these four lines back onto normal human metaphases. The generation of the translocation hybrids and of the chromosome 11q fragment hybrids is a necessary preliminary to determining whether a schizophrenia-predisposition gene SCZD2 is encoded at this site.

Introduction

A large Scottish pedigree (K26) has been described (St. Clair et al. 1990) in which major mental illness, classifiable as schizoaffective disorder, was seen to segregate with a balanced translocation, $t(1;11)(q43;q21)$. The family had been initially ascertained through a cytogenetic survey of mental hospitals and borstals (Jacobs et al. 1970). Subsequently (St. Clair et al. 1990) it was

found that 23 members of this family had psychiatric diagnoses conforming to the details set out in Research Diagnostic Criteria for mental and/or behavioral disorders (Spitzer et al. 1987). Thirty-four of the 77 individuals karyotyped carried the translocation. Sixteen of the 23 family members who had psychiatric diagnoses had major mental illness; two others, not analyzed cytogenetically, had committed suicide. The five remaining cases with psychiatric diagnoses but without the translocation did not have major mental illness (one had generalized anxiety, one had minor depressive disorder, and three were alcoholics). No physical dysmorphism or mental handicap had been observed in this carefully studied family.

Pedigrees with familial aggregations of common diseases, with both genetic and environmental components, are now used to unravel the specific genetic contributions to such disease, in the hope of gaining insight

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into the biochemical pathways affected, thus allowing us to think about therapy and prevention. A large pedigree where the disease segregates tightly with a cytogenetic abnormality is an ideal starting point for identifying the genetic component implicated at least in that family. Obviously, a cosegregating translocation may result in functional inactivation of a particular gene at or near the breakpoint site, but we are aware that the breakpoint may only be a closely linked marker for a dominantly acting disease-predisposing allele that happens to be present in this family, at a nearby locus. Two other, smaller families have been described with less clear-cut cosegregation of mental illness with two independent chromosome 11q translocations, one with chromosome 9 and one with chromosome 6 (Smith et al. 1989; Holland and Gosden 1990). The breakpoints were not accurately defined. However, their existence strengthened our interest in defining the exact site of the translocation breakpoint in pedigree K26.

Cytogenetic banding analysis was carried out on peripheral blood chromosomes from selected individuals, to confirm and refine the position of the breakpoint. Lymphoblastoid cell lines were set up from key family members, both translocation carriers and noncarriers. Genetic linkage analysis was carried out with available polymorphic markers in the region of the translocation, by using markers both from chromosome 1 and from chromosome 11. We expected to see suppression of recombination in the vicinity of the translocation but hoped to define more accurately the site of the breakpoint. Using markers which showed little or no recombination with the disease and the translocation in this family, we used chromosomal *in situ* hybridization to determine the position of the breakpoint, with the aid of suitable large insert markers. However, with a view to defining the breakpoint much more closely by positional cloning strategies, we also embarked on segregating the two translocation-derived chromosomes into separate somatic cell hybrids, using cell-surface marker selection strategies which we had developed for our work on the short arm of chromosome 11 (Seawright et al. 1988). The two independent pairs of mouse-human hybrids so produced showed TYR to be above the breakpoint and showed STMY1 to be below it. *In situ* hybridization confirmed the tyrosinase assignment to the derived chromosome 11 and showed the next most closely linked distal 11q marker, D11S388 (cosmid CJ52.4) (Julier et al. 1990), to be below the breakpoint. The chromosome 1 breakpoint was also defined with available markers and was found to be between D1S103 and D1S8. We then used DNA from the reciprocal

translocation hybrids MIS7 and MIS39 to set up and refine PCR-based analyses for a number of chromosome 11q loci.

Cell-surface marker analysis revealed early on that the two chromosome 11q markers MDU1 and MIC9 were, respectively, above and below the translocation breakpoint. Therefore we set out to produce stable chromosome-fragment hybrids, by X irradiation of the chromosome 11-only hybrid J1C14 (Jones et al. 1984), selecting for each marker separately. These were destined to be both a mapping and a cloning resource, for the production of new markers in regions defined by earlier markers.

Material and Methods

Genetic Analysis of Selected Family Members

DNA was available for linkage analysis, from 10 translocation carriers and 5 noncarriers in the pedigree. Individuals came from five branches of the family, each separated by several meiotic events. Analysis was carried out either by DNA blotting using informative restriction digests or by PCR followed by sizing of dinucleotide repeats on sequencing gels. Suitable markers were chosen above and below the breakpoint positions suggested by cytogenetic banding analysis on both translocation chromosomes: on chromosome 11—D11S97 (Jeffreys et al. 1988), TYR (Spritz et al. 1990; Giebel et al. 1991), D11S388 (Julier et al. 1990), and D11S35 (Litt et al. 1990); on chromosome 1—D1S81 (Nakamura et al. 1988), D1S103 (Weber et al. 1990), and D1S8 (Royle et al. 1988). Of these, D11S35, D1S103, and, latterly, TYR (Morris et al. 1991) were analyzed as dinucleotide repeats.

Cell Culture and Fusion

Peripheral blood lymphocytes were isolated from a number of cytogenetically normal and translocation-carrying members of the pedigree. In most cases, permanent B lymphoblastoid cell lines were produced by Epstein-Barr virus (EBV) transformation (van Heyningen, *in press*). Two cell lines, MAFLI and ROMAR, from translocation individuals with major mental illness have been used for most of the marker analyses. Culture of peripheral lymphocytes and EBV cell lines was in RPMI 1640 medium with 10% FCS. Preexisting cell hybrids were grown in appropriate medium: CF37, carrying an X-11 translocation chromosome (Mohandas et al. 1980) was grown in HAT medium; CF52 has an 11-16 translocation chromosome as its sole human chromosome complement and was grown in medium selective

for APRT (Koeffler et al. 1981). CF37 and CF52 were the gift of Dr. T. Mohandas. The CMGT hybrids E67.1 and E67.4 have been described elsewhere (Porteous et al. 1986) and have been analyzed in detail for short-arm markers (Porteous et al. 1987). They were grown in RPMI 1640 with 10% FCS.

The thioguanine-resistant, HPRT⁻ mouse cell lines used for fusion were Sp2/0-Ag4 (Shulman et al. 1978) and RAG (Klebe et al. 1970). Polyethylene glycol (PEG)-induced fusion, with MAFLI as the human parent, was carried out according to a method described elsewhere (van Heyningen, in press). The Sp2/0 hybrids, MIS series, grew in suspension and were subcloned by limiting dilution. The attached cell hybrids, MAR series, derived from RAG were subcloned by seeding about 100 cells into small Falcon flasks and transferring cell-surface marker-positive colonies into 2-ml wells 10–14 d after plating.

J1CI4 (a gift from Dr. Carol Jones; Jones et al. 1984) is a hamster cell hybrid carrying an intact chromosome 11 as its only human component. WG3H is an HPRT⁻ hamster cell line used as the recipient in X-irradiation fusions (Westerveld et al. 1971). Both of these cells were routinely grown in RPMI 1640 with 10% FCS.

Cell-Surface Marker Selection and Analysis

Hybrid cell selection for the expression of cell-surface markers expected on each "half" of the translocation chromosome was carried out under sterile conditions by using magnetic beads. The monoclonal antibodies used were in the form of sterile culture supernatant produced in our laboratory from hybridoma cells which we had bought or received as gifts. The hybridomas to detect MIC10 (on chromosome 1), MIC11, MIC4 (=CD44), MDU1, and MIC9 were, respectively, TRA2.10 (Andrews et al. 1985), from Dr. Peter Andrews; 163A5 (Woodroffe et al. 1984), from Dr. Frank Walsh; F10.44.2 (Goodfellow et al. 1982), from Dr. John Fabre; 4F2, from ATCC (HB-22); and 4D12 (Jones et al. 1984), from Dr. Peter Goodfellow.

Dynabeads M450 (sheep anti-mouse immunoglobulin conjugated) were used. The stock suspension is 4×10^8 beads/ml (30 mg/ml). The most efficient separation is expected if all the available second antibody sites are saturated with specific monoclonal antibody. One-milligram ($33 \mu\text{l} = 1.3 \times 10^7$) aliquots of beads were washed three times in sterile buffer (PBS and 1% BSA). They were then incubated with end-over-end rotation in 1 ml of each antibody in separate Eppendorf tubes for 10 min. The antibody was discarded after the beads were separated using the Dynabeads magnetic separa-

tor, and the coated beads were washed three times in buffer, to remove any unbound antibody.

A very small number (1–2 beads/cell) of coated beads were gently added to the cells suspended in PBS and incubated at room temperature with rotation of the samples, for 30 min. The beads, some with cells attached, were then separated magnetically and were washed three times in sterile serum-free medium and finally were gently resuspended in culture medium and were transferred to 2-ml wells.

For attached cells (the MAR hybrids) direct antigen-positive clone selection of attached cells is possible in a Falcon flask. The 30-min incubation of a 25-cm² flask with 10^4 coated beads/1 ml PBS is followed, without washing but with gentle rocking of the flask, by inspection, under the microscope, for bead-binding clones which can then be transferred into a 2-ml well by using separate sterile bacterial loops for each.

Cells with beads attached are handled as little as possible, to prevent mechanical damage. They continue to proliferate, and the beads are diluted out. Selection can be repeated and may be alternated with subcloning and quantitative fluorescence-activated cell sorter (FACS) analysis.

FACS analysis was carried out according to a method described elsewhere (Seawright et al. 1988; van Heyningen, in press), except that the cell sorter used was a Becton Dickinson FACSCAN. Anti-AFP monoclonal antibody was used as a negative control. The proportion of cell-surface marker-positive cells was estimated from the machine-derived plot of cell number against the log of fluorescence intensity.

Production of Cell-Surface Marker-selected X-Irradiation Hybrids

The chromosome 11-only hybrid J1CI4 (Jones et al. 1984) was used as the potential fragment donor, and the HPRT⁻ Chinese hamster cell line WG3H was used as the rodent fusion partner. A total of 2×10^7 log-phase J1CI4 cells were harvested by gentle trypsinization and were washed and resuspended in 2 ml of fresh medium containing 10% FCS in a bijou bottle which fits our gamma-irradiating cobalt source. A total of 16,000 rads were delivered to these cells. The cells were then washed in serum-free medium and fused with 1.7×10^7 freshly harvested and washed WG3H cells, by using our routine PEG fusion protocol. The fusion products were plated into $8 \times 75\text{-cm}^2$ flasks. HAT selection was implemented 16 h later. Two weeks after fusion, MDU1-positive and MIC9-positive clones were sought in the flasks, initially by using Dynabeads coated

with both antibodies. The positive colonies were picked with bacterial loops, were grown up as clones in 25-cm² flasks, and were reselected with the two antibodies separately and sequentially. Clones were FACS analyzed for all four chromosome 11-encoded cell-surface markers, and samples were cryopreserved at every stage. After repeated subcloning we obtained four relatively stable independent hybrid clones, two carrying each cell-surface marker in the absence of the others: WJX3 and WJX7 are 100% MDU1 positive; and WJX5 and WJX11 are 100% MIC9 positive.

Mitotic Chromosome Preparation

The techniques used for both lymphoid cells and somatic cell hybrids were essentially as described elsewhere (Fletcher, in press). For chromosome analysis by in situ hybridization, cell synchronization with fluorodeoxyuridine is recommended. Mitotic cell preparations can be stored in methanol:acetic acid (3:1) for at least 6 mo at -20°C. Spreads on slides were made as required.

Chromosomal In Situ Hybridization

The markers used for this analysis were suggested by preliminary linkage and hybrid cell results on determining flanking markers. Probes were labeled with biotin by nick-translation using routine methods (described in detail in Gosden 1990).

Slides were prepared 3–4 d before the hybridization was carried out. The hybridization protocol was as described by Gosden (1990).

Detection was essentially as described by Pinkel et al. (1986), using (a) avidin-FITC (Vector Labs) as reporter and (b) linking layers of FITC with biotinylated anti-avidin. The slides were counterstained with propidium iodide and were mounted in Citifluor antifade:glycerol. They were examined on a Leitz Ortholux II microscope equipped with Ploemopak fluorescence filters, and suitable metaphase spreads were scanned and stored with the BioRad Lasersharp MRC 600 confocal laser scanning system attached to the same microscope.

The markers used to define the breakpoint were a lambda phage clone of tyrosinase and the next distal marker, cosmid CJ52.4. The latter defines the locus D11S388 (Julier et al. 1990).

Cytogenetic Analysis of X Irradiation-Fragment Hybrids

The human DNA content of these hybrids was analyzed in several different ways. Mitotic chromosome preparations from the subcloned stabilized hybrids

were hybridized with biotinylated human Cot1 DNA (GIBCO BRL), and signal was developed with a single layer of avidin-FITC and was counterstained with propidium iodide in Citifluor by the method above. Hybridization conditions used were as described by Gosden and Hanratty (1991b).

The origin of the human fragments in these hybrids was also determined by chromosome painting. For this the hybrid cell DNA was subjected to interrepeat sequence (IRS)-PCR using several primers for the human Alu and L1 interspersed repeated sequences (table 1; Gosden and Hanratty 1991a; Dorin et al. 1992). The PCR products were labeled with biotin by nick-translation and were used as a probe for in situ hybridization back onto normal human mitotic spreads of relatively extended chromosomes. Considerable suppression, by adding 500 ng of unlabeled human Cot1 DNA to 50 ng labeled IRS-PCR product for each slide, was required to give chromosome-specific results. Hybridization and detection were essentially as described above for specific marker in situ analysis.

Marker Analysis

DNA was prepared, by standard procedures described elsewhere (van Heyningen, in press), from frozen cell pellets, for all of the cell lines. Most marker analysis was by PCR, and the products were analyzed on agarose (1.5% for fragments of 500 bp). DNA blot analysis was carried out for the chromosome 1 probes D1S8 and D1S81.

Designing and Setting Up PCR Assays for Chromosome 11 Markers

The aim was to set up PCR-based assays to quickly analyze somatic cell hybrids for the presence of human chromosome 11 markers on both mouse and hamster backgrounds. Except for a few short-arm markers, we concentrated on well-spaced long-arm markers. Where no published primer sequences were available, we designed our own, either from published gene sequences or, in the case of D11S388, D11S385, and D11S351, from data obtained by sequencing a fragment of the insert from cloned probes. In order to try to keep the PCR human-specific, primers were designed from untranslated regions or introns, wherever such information was available. Theoretical conditions were calculated for each primer pair and then were refined by experimentation. Final primer sequences and annealing temperatures are shown in table 1. For chromosome 1 markers—renin (REN) (Theune et al. 1991) and

Table 1

PCR Primers Used to Detect Genes in Somatic Cell Hybrids and for IRS-PCR for Painting Human DNA Content of Hybrid Cells back to Normal Karyotype

Repeat	Primer(s)	Annealing Temperature (°C)	Fragment Size (bp)	Reference (remarks)
HBB	{ 5' ATG GTG CAC CTG ACT CCT GAG G 3' } { 5' GCC ATC ACT AAA GGC ACC GAG C 3' }	65	355	In-house design
D11S87	{ 5' CCC TGG AAA CAC TTT CTG CC 3' } { 5' GGC TGG GTT GGA GGC AAG G 3' }	65	120	Lewis et al. 1988; in-house sequence and design
TYRL1	{ 5' ACA ATA TGT TTC TTA GTC TG 3' } { 5' TGG TAA CAC TAG ATT CAG C 3' }	51	260	Giebel et al. 1991 (exon 4 oligos P1 and P2; <i>MspI</i> to distinguish from TYR)
PGA	{ 5' GCA TCT CTG ACA CCA ATC AG 3' } { 5' TGG AGA AGA GAC AGA TGG AG 3' }	54	188	W. Cookson, personal communication
MDU1	{ 5' TCT TCA AAG CCT CTG CAG TAC C 3' } { 5' CTC ATC TCC AAC CTG TCT AAC C 3' }	57	859	Gottesdiener et al. 1988; in-house design (nt -1023 to -174)
FGF4	{ 5' GAT GAG TGC ACG TTC AAG GAG 3' } { 5' CAG AGA TGC TCC ACG CCA TAC 3' }	62	542	Yoshida et al. 1987; in-house design (nt 4161 to 4703)
GST3	{ 5' GGG AGG GAT GAG AGT AGG ATG 3' } { 5' GGA GGT TCA CGT ACT CAG GGG 3' }	60	450	Cowell et al. 1988; in-house design (exons 5-7)
TYR	{ 5' GCA AGT TTG GCT TTT GGG GA 3' } { 5' CTG CCA AGA GGA GAA GAA TG 3' }	55	512	Giebel and Spritz 1990 (exon 1 oligos)
D11S388	{ 5' CAT CCA CGC TGT TGG TCT GC 3' } { 5' CAG TAA GAA CCA AAG AAA GGT TAC 3' }	51	118 (variable)	Julier et al. 1990; in-house sequence and design
STMY1	{ 5' CAG TTT TCT CCT CTA CCA AGA C 3' } { 5' ACT GGC TTT ACT TAG CTC TAT G 3' }	50	300	Sirum and Brinkerhoff 1989; in-house design (nt -300 to -4)
CLG	{ 5' AGT CAG TAC AGG AGC CGA ACA G 3' } { 5' GGA GAA AAG CTG TGC ATA CTG G 3' }	59	517	Angel et al. 1987; in-house design (nt -434 to +83)
D11S385	{ 5' TTT TAT AGG GAC AGG ATC TTG C 3' } { 5' GGC TGT ATA ATC TTG TGT TCT C 3' }	54	286	Julier et al. 1990; in-house sequence and design
NCAM	{ 5' TGG AAA TCT CTT CCA AAC ATC GGA G 3' } { 5' AAT TAG AAC TTT GGA GAG GGA TGG G 3' }	55	179	Barton et al. 1990 (nt -848 to -663; designed by G. Gillett)
DRD2	{ 5' GAG GCC CTC TCA CTG ACA C 3' } { 5' AGT GCA GGG CCC TGC TGG A 3' }	55	390	R. Todd, personal communication (exon 2)
D11S351	{ 5' CTT GGG TAG CTG GTA CTA CAG G 3' } { 5' AGG TCA CTA CAC ATC AAA ACA GC 3' }	59	209	Julier et al. 1990; in-house sequence and design
THY1	{ 5' CAG AAG GTG ACC AGC CTA ACG 3' } { 5' CTG AGC ACT GTG ACG TTC TGG 3' }	40	324	Cotter et al. 1989 (with slight modification)
D1S103	{ 5' ACG AAC ATT CTA CAA GTT AC 3' } { 5' TTT CAG AGA AAC TGA CAT GT 3' }	55	>85 (variable)	Weber et al. 1990

(continued)

Table 1 (continued)

Repeat	Primer(s)	Annealing Temperature (°C)	Fragment Size (bp)	Reference (remarks)
5' End Alu } Consensus }	{ 5' AAAGTGCTGGGATTACAGG 3' } { 5' GTGGCTCACGCCTGTAATCCC 3' }	60	Various	Dorin et al. 1992
3' End Alu } Consensus }	{ 5' GTGAGCCGAGATCGCGCCACTGCACT 3' } { 5' TGCCTCCAGCCTGGGCAACA 3' }	60		
L1H	5' CATGGCACATGTATACATATAGTAAC(A/T)AACC 3'	60		
HumanL1A ...	5' CACAGGAAGGGGAACATCACA 3'	60		
HumanL1B	5' GGGGAGGGATAGCATTAGGAG 3'	60		

D1S103 (Weber et al. 1990)—published PCR conditions were used.

A problem which had to be overcome in the tyrosinase analysis was the existence of the tyrosinase-like gene (TYRL1), which was described in detail while the present work was in progress (Giebel et al. 1991). This allowed us to resolve TYRL1 from TYR by using primers to TYR exon 4, which is common to both loci; but, after *MspI* or *HpaII* digestion of the PCR product, TYR gives rise to 120-bp and 140-bp fragments, while the TYRL1 products are 29 bp, 91 bp, and 149 bp (data not shown). For the detection of TYR alone, primers for exon 1 were used (table 1).

Results

Genetic Analysis

With the more distant but highly informative chromosome 11 breakpoint-flanking markers D11S97 and D11S35, two and three obligate recombinants, respectively, were found among the 10 translocation carriers. For the closer but less informative flanking markers TYR and D11S388, no recombinants were found, even when DNA was reanalyzed for the recently described TYR dinucleotide-repeat polymorphism (Morris et al. 1991). On chromosome 1 several recombinants are seen with the highly informative distal marker D1S8, but none was found with the proximal markers D1S103 or D1S81—both of which are highly polymorphic reference markers. These results were emerging as the cell hybrid analysis and the in situ hybridization were getting under way and gave us an idea of the markers to study, especially for the latter technique.

Marker Analysis of the Hybrid Cell Panel

The stabilized, repeatedly subcloned, cell-surface marker-selected translocation hybrids were analyzed mainly by PCR, once segregation of the two derived chromosomes from their normal homologues had been ascertained by cell-surface marker expression (fig. 1). The X irradiation-fragment hybrids were screened in the same way. Included in the panel for analysis, both by PCR and for cell-surface markers, were potentially useful preexisting translocation hybrids and earlier CMGT fragment hybrids. High-molecular-weight DNA was used for all analyses.

The PCR primers shown in table 1 were used to analyze the complete hybrid cell panel, including some earlier CMGT chromosome-fragment hybrids. The complete data set is shown in table 2, where the position of the translocation breakpoint is clearly defined by hybrids MIS7 (=MAR12) and MIS39 (=MAR1), and the marker analysis of the fragment hybrids gives an idea of what chromosome 11q regions are present for future marker isolation.

Molecular Cytogenetic Analysis of the X Irradiation-Fragment Hybrids

The human DNA content of the WJX hybrids was analyzed by in situ hybridization using human Cot1 DNA. Figure 2 shows the distribution of the chromosome 11 fragments in each hybrid. In three of four cases there appears to be a single block of human DNA, but in WJX7 three separate blocks are seen, although all three are located on the same recipient chromosome. The appearance of the single extended region in WJX5 is nonuniform.

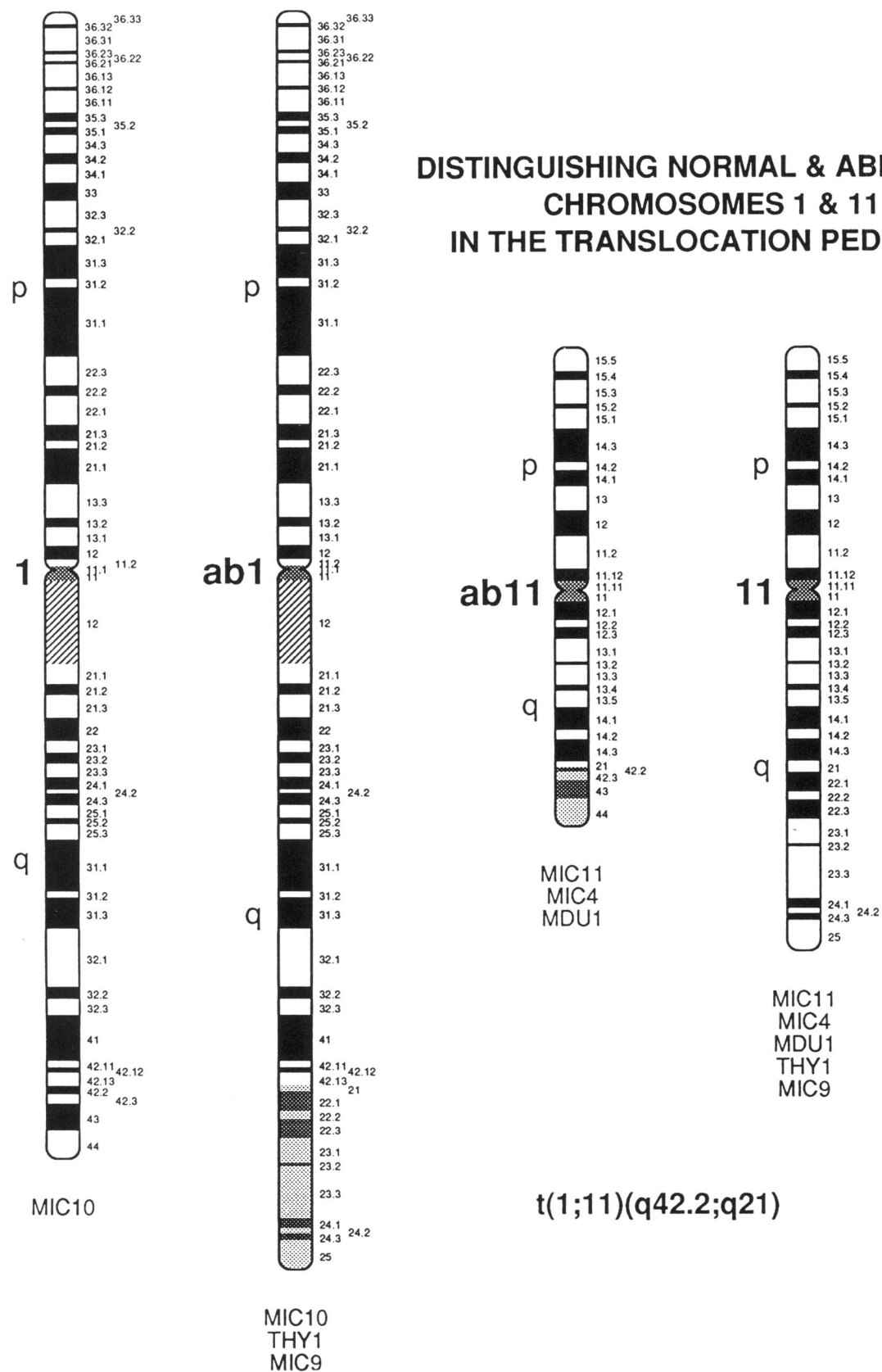


Figure 1 Strategy for distinguishing hybrid cells carrying normal and derivative chromosomes 1 and 11, using cell-surface marker selection.

Table 2**Marker Analysis of Translocation, X Irradiation, and CMGT Hybrids**

	STATUS OF CELL LINE(S) ^a								
	MIS7 and MAR12	MIS39 and MAR1	CF37 and CF52	WJX3	WJX5	WJX7	WJX11	E67.1	E67.4
CHR 11 markers:									
HBB	—	+	ND	—	+	—	—	+	—
D11S87	—	+	ND	—	—	—	—	ND	ND
MIC11	—	+	—	—	—	—	—	+	+
CD44	—	+	—	—	—	—	—	—	+
TYRL1	—	+	—	+	+	—	—	—	+
PGA	—	+	—	+	—	+	—	+	—
MDU1	—	+	—	+	—	+	—	—	—
FGF4	—	+	—	+	—	—	—	—	—
GST3	—	+	—	+	—	—	—	+	+
TYR	—	+	+	—	—	+	+	—	+
D11S388	+	—	+	—	—	—	+	—	—
STMY1	+	—	+	—	+	+	+	—	+
CLG	+	—	+	—	+	+	+	—	+
D11S385	+	—	+	—	+	+	+	—	+
NCAM	+	—	ND	—	—	+	+	ND	ND
DRD2	+	—	ND	—	—	+	+	ND	ND
D11S351	+	—	+	—	+	—	+	—	—
THY1	+	—	+	—	+	—	+	ND	ND
MIC9	(+)	—	+	—	+	—	+	—	—
CHR 1 markers:									
MIC10	+	—	ND	ND	ND	ND	ND	ND	ND
REN	+	—	ND	ND	ND	ND	ND	ND	ND
D1S103	+	—	ND	ND	ND	ND	ND	ND	ND
D1S8	—	+	ND	ND	ND	ND	ND	ND	ND

^a A plus sign (+) denotes presence of marker; and a minus sign (—) denotes absence of marker. ND = not determined.

Chromosome painting onto a normal human metaphase spread was also carried out to study directly the chromosome 11 regions from which the human fragments were derived. Figure 3 reveals an apparently single contiguous region of chromosome 11q in WJX3, WJX7, and WJX11. Three distinct regions, two on the short arm and one on the long arm, are seen only in WJX5, as expected on the basis of marker analysis (table 2). Marker analysis would suggest the presence of discontinuity in WJX7 too. Cot1 analysis of the hybrid showed three separate blocks of human DNA in WJX7, and the results in table 2 reveal two gaps—first, in the HSTF1, GST3 region and, second, around D11S388. This suggests that two small interstitial deletions have interrupted contiguity, and the three separate blocks of human DNA seen in the hybrid cell line may represent the separate insertion of each contiguous segment. However, as the gaps are small, the human DNA con-

tent of WJX7 appears as a single block when painted back onto normal human chromosomes.

In Situ Hybridization to Determine the Site of the Translocation Breakpoint

Figure 4 shows the results with the flanking markers on mitotic chromosomes prepared from peripheral leukocytes of translocation carrier and affected patient ROMAR. Figure 4a shows the breakpoint-distal marker D11S388 hybridizing to the large derived chromosome 1 and to the normal chromosome 11 homologue, and figure 4b shows the TYR signal on the normal chromosome 11 and on the visibly smaller, derived chromosome 11.

Discussion

Schizophrenia and related major mental illness are very common in the general population (Gottesman

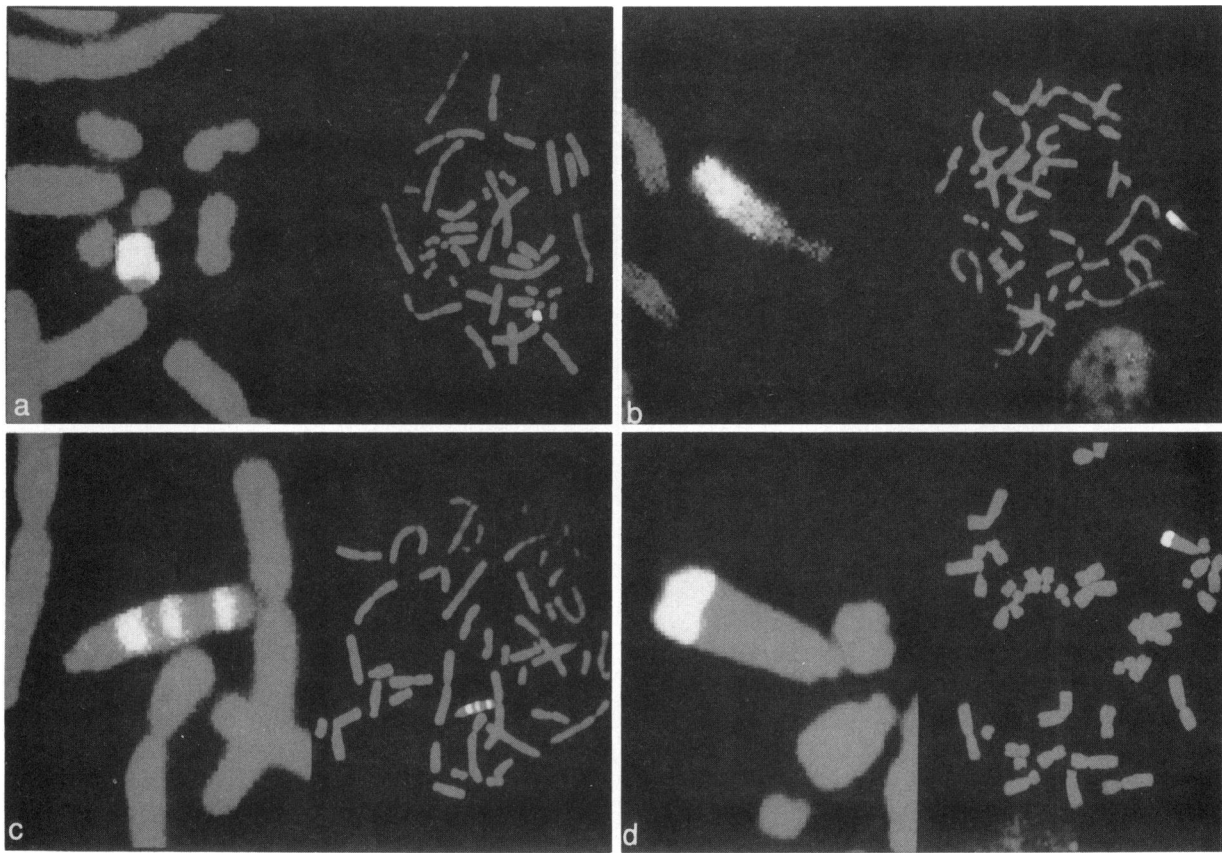


Figure 2 Human DNA content of the WJX hybrids, revealed by in situ hybridization with Cot1 fraction of human DNA. *a*, WJX3. *b*, WJX5. *c*, WJX7. *d*, WJX11. For each cell line a representative mitotic spread is shown on the right, with an enlarged view of the human fragment-bearing chromosome in the left panel.

and Shields 1982). The etiology of these diseases is not understood but is almost certainly heterogeneous. Social, environmental, and genetic components interact to cause disease. Twin studies point to a major genetic contribution (Gottesman and Shields 1982), which is, however, likely to be due to several different interacting genes. Any insight into the physiological/biochemical abnormalities which are involved in the onset of disease may give a lead in designing improved therapy and, perhaps, in devising preventive measures. Understanding the underlying pathology in one form of the disease may lead to other candidate genes or to environmentally alterable targets.

With this philosophy in mind, we set out to identify, as a pointer to one candidate genetic component, the translocation breakpoint which cosegregates closely with schizophrenic illness in this large kindred. We have succeeded in showing that the breakpoint lies between two adjacent genetically linked markers, TYR

and D11S388, initially placed 4 cM apart (C. Julier, unpublished results) but most recently estimated to be about 7.8 cM apart at 11q14-q21 (NIH/CEPH Collaborative Mapping Group 1992). The DNA distance between two such markers is impossible to predict, since the relationship between genetic and physical distance is not constant, so that in part of the nearby 11q13 MEN1 region 1 cM corresponds to only 300 kb (Janson et al. 1991). The next step is to identify the exact site of the breakpoint, with the aid of further markers in the TYR-D11S388 interval. With this in mind we segregated the reciprocal translocation chromosomes into stable mouse-human hybrids, for quick assignment of any newly isolated markers. We have also produced the four WJX series of X-irradiation hybrids carrying 11q fragments selected with cell-surface markers known to map above and below the 11q21 breakpoint. Of these, WJX11 in particular reveals a contiguous block of markers which appear to cross the transloca-

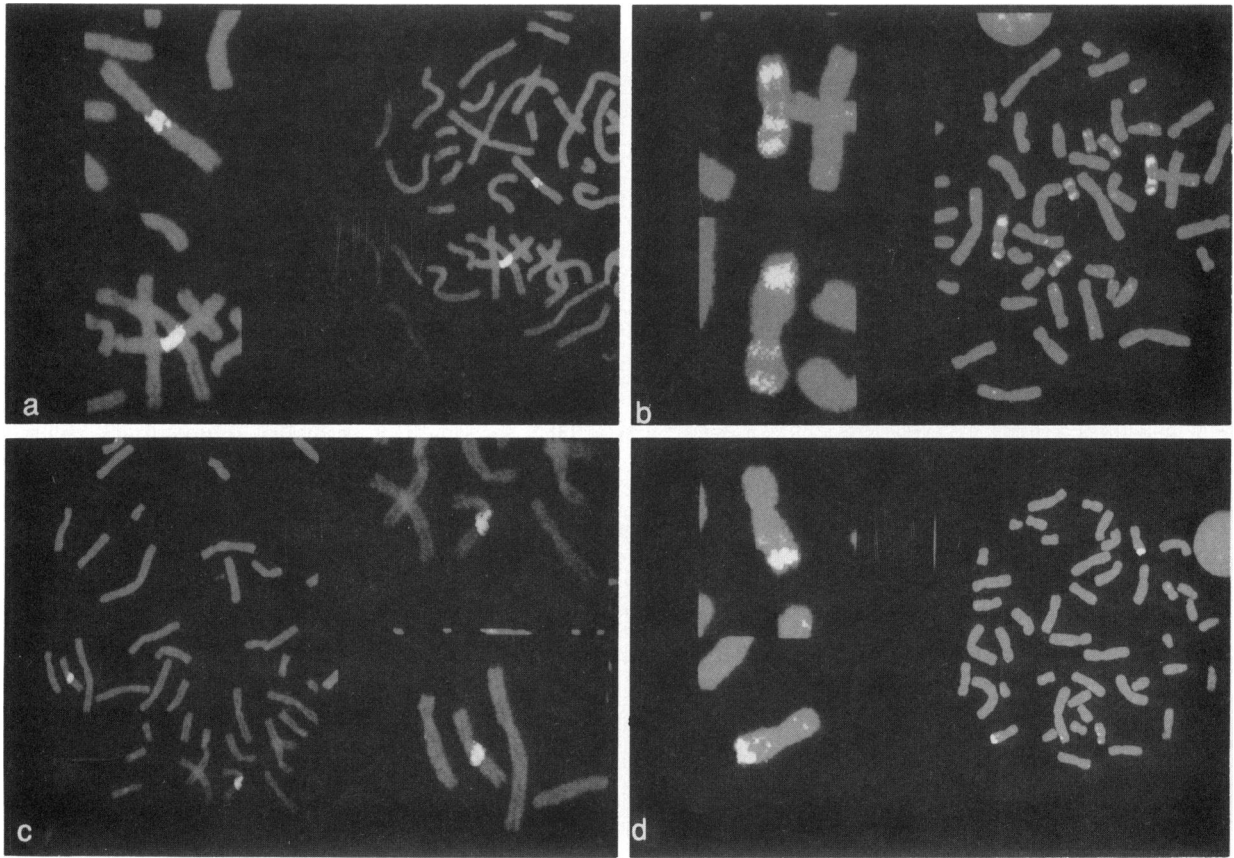


Figure 3 Human DNA from each WJX hybrid, isolated by Alu-PCR and painted back onto normal human mitotic spreads. *a*, WJX3, showing a subcentromeric block of DNA on 11q. *b*, WJX5, revealing the presence of three distinct blocks, from 11pter, 11p11-p12, and 11q23-q24 (the degree of Cot1 suppression was not quite sufficient in this case, showing hybridization to some other chromosomes). *c*, WJX7, showing a single relatively centromere-proximal block on 11q. *d*, WJX11, with a more distal 11q block of DNA. Each chromosome 11 homologue is shown enlarged separately in a small panel.

tion breakpoint. We cannot be certain of the integrity of the DNA without PFGE analysis, since, for example, in CMGT hybrids (of which E67.1 and E67.4 are representative), we almost invariably find the human DNA components to be rearranged when looked at by long-range techniques (Bickmore et al. 1989). Alu-PCR painting similarly reveals discontinuity in some chromosome-fragment hybrids (Dorin et al. 1992). Nevertheless, such hybrids provide an excellent source of new markers for the chromosome regions indicated to be present by the preexisting markers (Porteous et al. 1987; Bickmore et al. 1989). However, to narrow marker isolation more closely to the region of interest, we propose to try coincidence sequence cloning (Brookes and Porteous 1991)—for example, between MIS39 (=MAR1) and WJX11, which carry overlapping human fragments on a mouse cell background and

hamster cell background, respectively. Using this technique should allow us to isolate new markers solely for the human region which the two cells have in common, which is around the translocation breakpoint. Other approaches in progress that are seeking to produce markers for the region of interest include (*a*) the production of microdissection libraries and (*b*) the isolation of TYR and D11S388 yeast artificial chromosome (YAC) clones, followed by YAC walking between these clones. New markers produced are quickly mapped onto the translocation hybrids MIS7, MIS39, and CF37. Markers present in both MIS39 and CF37 or WJX11 are automatically in the region of interest. Candidate YAC clones for crossing the breakpoint can be identified by in situ hybridization (Breen et al. 1992) onto the translocation cell line MAFLI.

In the process of producing and analyzing the so-

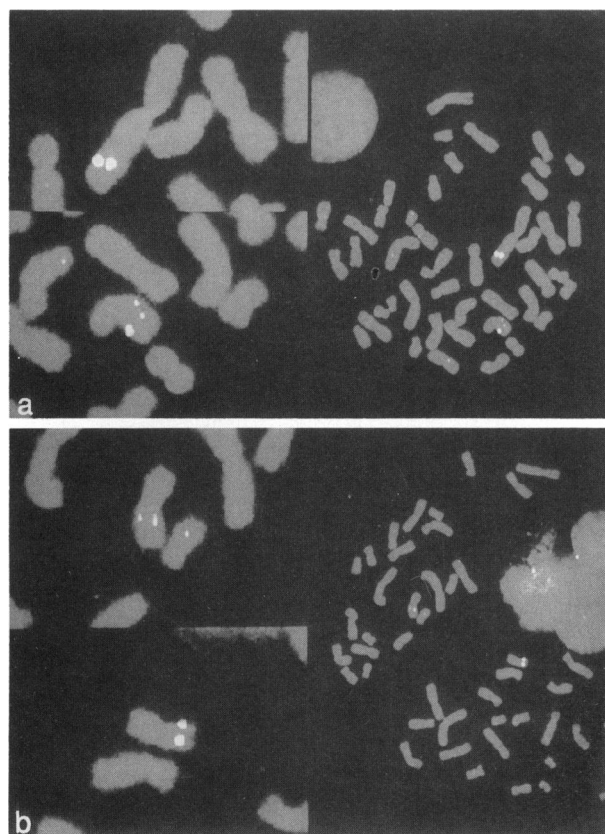


Figure 4 In situ hybridization of the two adjacent flanking markers on mitotic spreads from an affected translocation carrier. *a*, D11S388, which hybridizes to the derived 1 (upper left) and to the normal 11 homologue (lower left). *b*, TYR signals, seen on the normal 11 homologue (upper left) and on the truncated-looking derived 11 (lower left).

matic cell hybrids described, we have contributed to the mapping effort on chromosome 11q. We have devised human-specific PCR assays for a number of gene-specific markers and have developed new sequence-tagged sites for the D11S351, D11S385, and D11S388 (Julier et al. 1990) loci. The localization of the FACS-selectable cell-surface marker MDU1 (Seawright et al. 1988), which is also the cloned gene 4F2 (Gottesdiener et al. 1988), has been refined to the region proximal to TYR but deleted in J1-44 on 11q (Jones et al. 1984; Tanigami et al. 1992). It is interesting to note that, although there is considerable fragmentation of the retained chromosome fragments in WJX5 and WJX7, markers known to be closely linked, such as the CLG/STMY1/D11S385 complex and NCAM/DRD2 (McConville et al. 1990), are not separated. These re-

sults suggest that such fragment hybrids will be useful for short-range mapping.

Linkage studies of chromosome 11q markers with schizophrenia have recently been carried out (Muir et al. 1991). Interest in a possible disease association with this chromosomal region has been influenced by (1) the finding of three 11q21-q23 translocations (Smith et al. 1989; Holland and Gosden 1990; St. Clair et al. 1990), (2) the existence of several published cases of schizophrenia in albino patients, implicating the tyrosinase region (Baron 1976; Clarke and Buckley 1989), and (3) the presence of possible candidate loci, such as the dopamine D₂ receptor (Grandy et al. 1989; Seeman et al. 1989). Although the results of these studies generally have been negative, it is not possible to exclude the involvement of a major locus SCZD2 in this region, in as many as 20% of cases, making the isolation of genes from the breakpoint region an important approach for identifying specific schizophrenia-predisposition genes which may be implicated in only a proportion of cases.

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